

BLOOD PRACTICAL 3

Blood groups, Clotting Time and Bleeding Time

Aims of the Practical

To determine

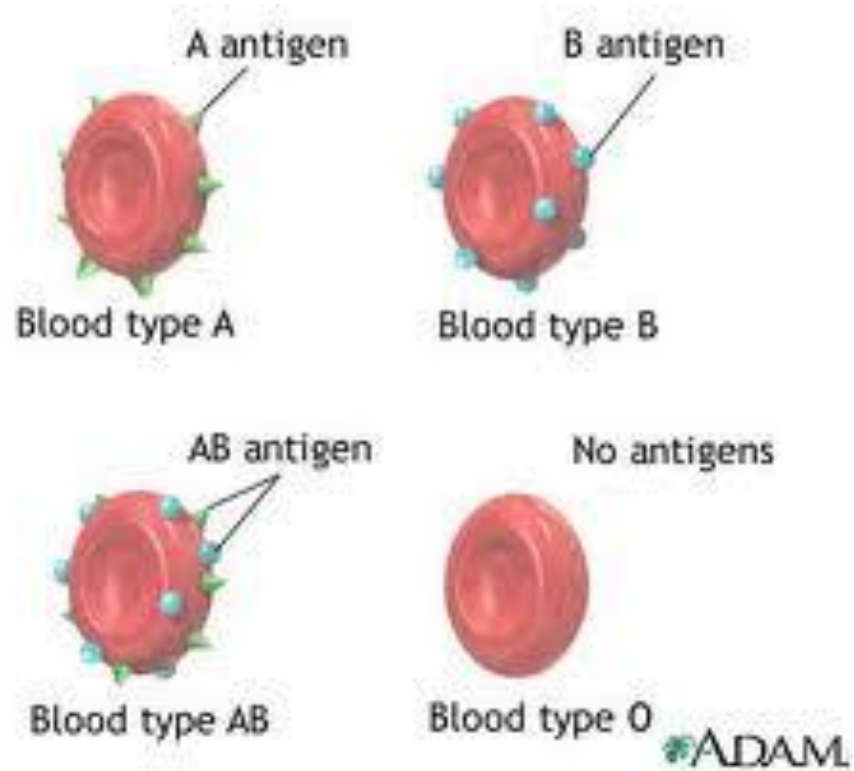
- 1. blood groups.**
- 2. clotting time.**
- 3. the bleeding time.**

ABO Blood groups

- **ABO System**

- **Group A: antigen A on RBC surface antiB in plasma**
- **Group B: Antigen B on RBC membrane AntiA in plasma**
- **Group AB: Antigen A and B on RBC membrane NO antibodies in plasma**
- **Group O : NO antigen on RBC membrane both AntiA and AntiB in plasma**

Blood group Antigen



Rhesus Blood group

Rhesus antigen D

- 1. Rhesus positive (Rh+ve): Antigen D on RBC (96-98%)**
- 2. Rhesus negative (Rh-ve): NO Antigen D on RBC (2-4%)**

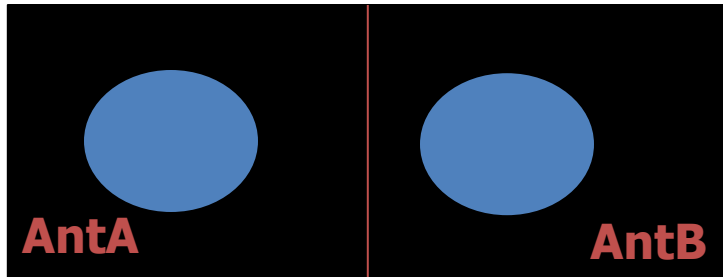
Material and methods

- **High titer anti-A, anti-B and anti-D sera**
- **A microscope**
- **A grease pencil**
- **Microscope slides**

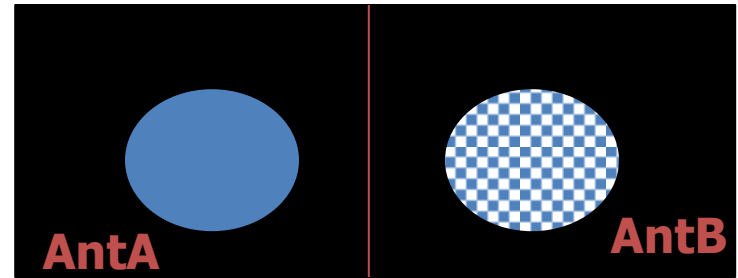
Procedure

- Prick a finger and place one drop of blood in each of the compartments A, B and D. These are clearly labelled on the microscope slides provided.
- Quickly add a drop of anti-A, anti-B and anti-D sera to compartments A, B and D respectively.
- Mix the serum with the drop of blood by moving the slides gently to and fro for a minute or two.
- Examine the mixtures for signs of RBC agglutination or clump formation.

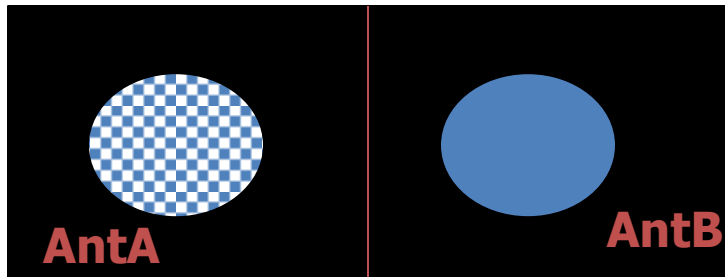
Blood Group O



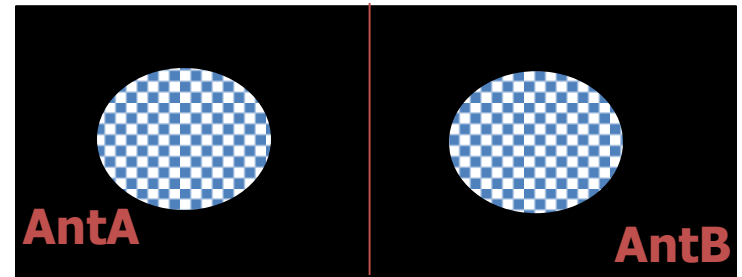
Blood Group B



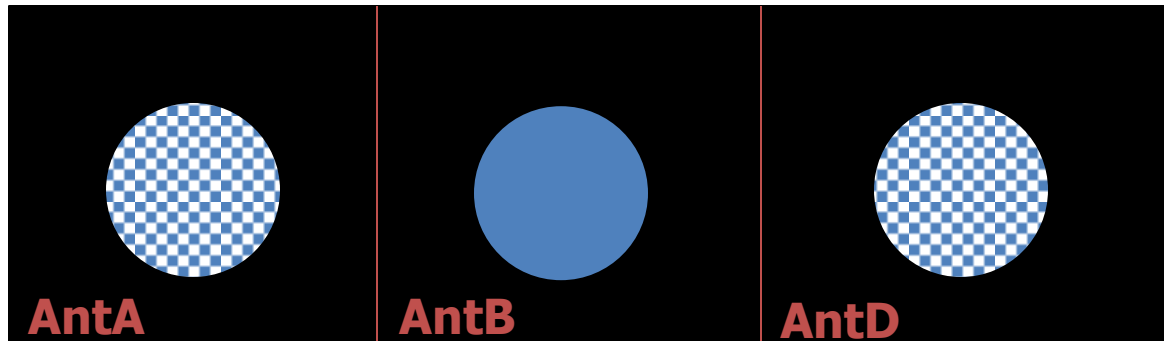
Blood Group A



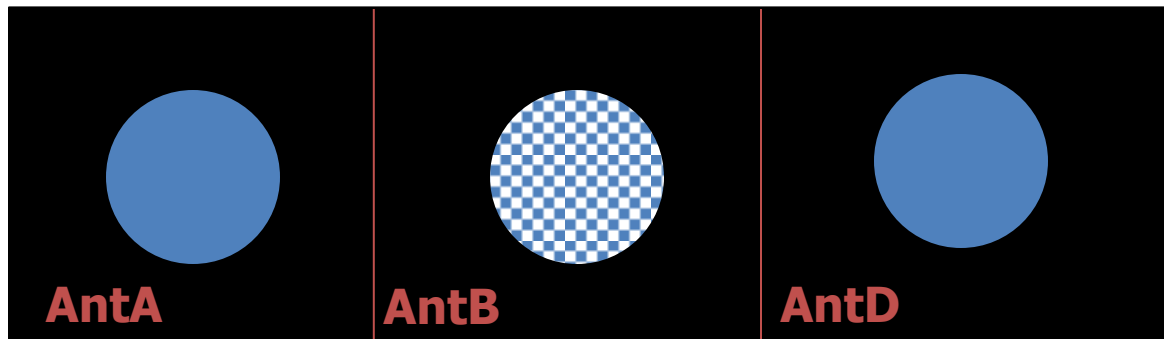
Blood Group AB



Blood group A+ve



Blood group B-ve



Clotting Time

Clotting Time

- **The time required for blood to form a clot.**
- **The normal coagulation time in glass tubes is 5 to 15 minutes.**
- **The whole blood clotting time is a rough measure of all intrinsic clotting factors in the absence of tissue factors.**
- **This simple test has been used to diagnose hemophilia.**
- **Its chief application is in monitoring anticoagulant therapy.**

Reagents and apparatus

- **Capillary tubes of uniform size**
- **A petri-dish**
- **Alcohol swabs**
- **Cotton wool**
- **Plasticine**
- **A water bath set at 37°C**

Procedure

- **Clean finger with alcohol swap, Prick it with lancet and note the time that the prick is made.**
- **Wipe away the first drop of blood. Then while the blood is still flowing freely place one end of a capillary tube in the blood. Holding the tube horizontally let it fill by capillary action, Fill more than one tube**
- **Close the end of the capillary tube with plasticine. Place the tube in the water bath.**

Procedure

- **Two minutes after making the puncture, break a capillary tube and separate the two halves slowly.**
- **Repeat the procedure at 30 second intervals with the remaining tubes.**
- **When the blood forms a continuous thread-like clot between the broken ends of the tube, the end-point has been reached, Note the time.**
- **The time from pricking the finger to the appearance of the clot is the clotting time**



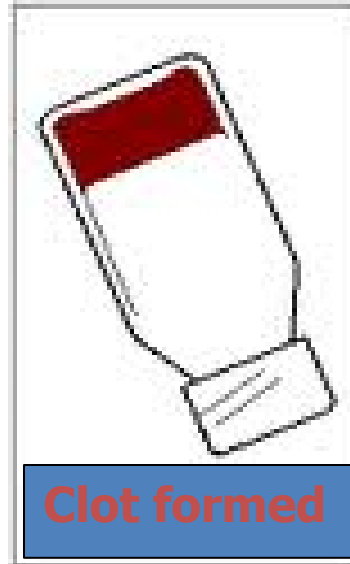
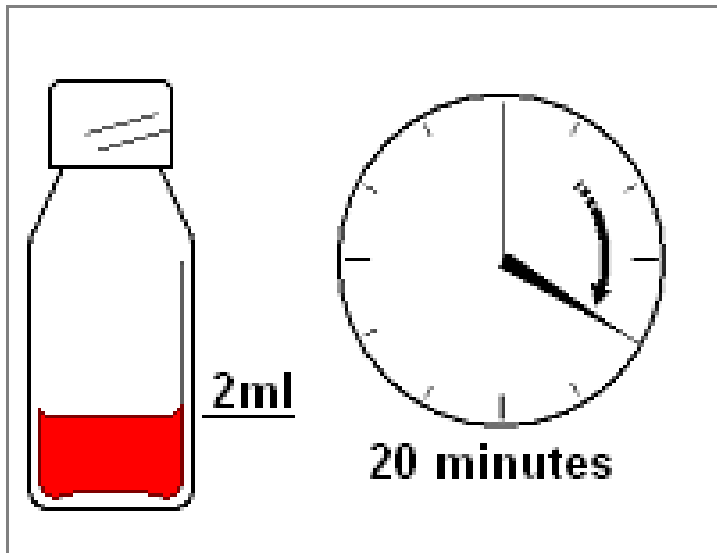
Results

- Usually the clotting time measured by this method is in the range 5-15 minutes.
- Prolong clotting time seen in deficiencies in the intrinsic coagulation pathway
- Example: haemophilia due to deficiency of Factor VIII (8)

Clotting time using test tube method

- **Place 2 ml blood into non heparnized test tube incubated in water bath**
- **Every 30 second invert gentle to check for clot formation**
- **Time from pricking finger to clot formation is clotting time**

Test Tube Clotting Time



Bleeding Time

Bleeding Time

- **Bleeding time** is a test of platelet function
- The time it takes for bleeding to stop (time for a platelet plug to form).
- The template bleeding time is used when the test is performed by standard template method

Reagents and apparatus

- **Blotting paper**
- **A stop-watch**
- **A stylette to prick an ear lobe**
- **Alcohol swabs**

Procedure

- Clean the lobe of the ear with an alcohol swab.
- When it is dry, make a single puncture with a stylette (about 3mm deep). Note the time at which the puncture is made.
- The skin of the ear should not be touched once the puncture has been made until the experiment is over.

Procedure

- Apply a piece of filter paper to the blood-drop every 30 seconds until the bleeding stops.
- The bleeding time estimated by this method of a normal subject is within the range of 2-5 minutes.

Bleeding Time



The standardized template method

- A sphygmomanometer cuff is applied to the subject's arm and inflated to 40mmHg.
- The volar surface is cleaned with 70% alcohol.
- A sterile metal template with a linear slit (11mm long) is pressed firmly against the skin.
- A scalpel blade, with a guard, is carefully introduced so that it protrudes 1mm through the template slit. An incision, 1mm deep and 9mm long can then be made.
- Blood is gently, but completely removed with filter paper at 15 second intervals until the bleeding stops.
- Normal bleeding times determined with this method are in the range 2.5-9.5 minutes.

Bleeding Time



Two incisions are made
and the time for clotting
to occur is recorded

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Clinical Application

- **Bleeding time is affected by platelet function, certain vascular disorders and von Willebrand Disease, thrombocytopenia,**
- **Aspirin can prolong bleeding time significantly.**
- **People with von Willebrand disease usually experience increased bleeding time, as von Willebrand factor is a platelet agglutination protein.**
- **Normal values fall between 2 – 9 minutes depending on the method used.**

Objectives

At the end of this lesson the student should be able to:

- 1. Understand and practice the method used in determining blood groups (ABO and Rhesus (Rh) systems), and be able to explain their importance in blood transfusion.**
- 2. Determined his own Bleeding and clotting time compared to normal range of values expected for the bleeding and clotting time.**
- 3. Recognize the importance of bleeding time and clotting time in haemostasis.**